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STUDY OF RHAMNOLIPIDS CYTOTOXICITY, INHIBITORY EFFECT ON SOME MICROORGANISMS AND APPLYING IN FOOD PRODUCTS

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ABSTRACT

The local isolate Pseudomonas aeruginosa P.a.28that we isolated it from the contaminated soil of oil field of NahranUmar in Basra governorate in a previous study, was the best producer of biosurfactant (rhamnolipids) with high emulsification activity. Testing of inhibitory effect of this bio surfactant toward some of G^+ ,G^- bacteria and yeasts appeared, that the maximum antibacterial activity was against Bacillus subtilis with a diameter of inhibition zone 30 mm , while the minimum antibacterial activity was against Micrococcus roseus with diameter of inhibition zone 10mm .Also antifungal activity against the tested yeasts Candidaalbicans and Rhodotorulasp. were found with inhibition zones 27 mm and 17mm respectively. Results showed that rhamnolipids did not cause cytotoxicity when tested with human blood. So we finallytestedit in some food products such as ice cream, loaf and mayonnaise, the results showed that the sensory evaluation of samples with rhamnolipids had significant differences in the taste, flavor, texture and external appearance propertiescomparing with the control.

KEYWORDS: Pseudomonas aeruginosa, Rhamnolipids, Cytotoxicity, Inhibitory Effect, Applications

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Original Article

INTRODUCTION

Biosurfactants are amphiphilic compounds produced on living surfaces, mostly microbial cell surfaces or excreted extracellularly and contain hydrophobic and hydrophilic moieties that reduce surface tension and interfacialtension between individual moleculesat the surface and the interface respectively. Biosurfactants are produced by a wide variety of bacteria, yeasts and filamentous fungi. The most advantage of biosurfactants when compared to synthetic surfactants is their ecological acceptance owing to their low toxicity and biodegradable nature (Karanth,*et al.*1999). Applications could be found in the food, pharmaceutical, cosmetics and specialtychemical industries (Fiechter,1992).Biosurfactants have many potential industrial and environmental applications related to emulsification, foaming, detergency, wetting, dispersion and solubilisation of hydrophobic compounds (Banat, *et al.*,2000; Destgheilb, *et al.*, 2008).In addition to surface activity, Biosurfactants are useful as antibacterial, antifungal and antiviral agents, and they also have the potential for use as major immune modulatory molecules and adhesive agents (Rodrigues, *et al.*, 2006). Rhamnolipids is one of type of glycolipids, in which one or two molecules of rhamnose are linked to one or two molecules of hydroxydecanoic acid while the OH group of one of the acids is involved in glycosidic linkage with the reducing end of the rhamnose disaccharide, the OH group of the second acid is involved in ester formation (Karanth,*et al.*1999).Rhamnolipids is produced by*Pseudomonas aeruginosa*, a G^- bacteria,aerobic, with (0.5-1×1.5-3.0)µm,motile with a polar flagellum, no spores forming, oxidase positive and hemolysis (Garrity*et.al.*,2001).

P. aeruginosa can degrade the aromatic hydrocarbons by producing biosurfactant especially rhamnolipids (Wikipedia,2008).

Abalos,*et.al*.,(2001) investigated the antibacterial and antifungal activity of rhamnolipids. AlsoHaba*et.al*.,(2003) revealed the antibacterial and antifungal activity and minimum inhibitory concentration (MIC) of rhamnolipids, they observed that MIC was between (4-75) mg/ml to inhibit *Serratiamarcescens* , *Klebsiella pneumonia* , *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Penicilliumfuniculosum* , *Gliocladiumvirens* and *Fusariumsolani*.

There are many industrial application for biosurfactantssuch as chemical industries, pharmaceutical and foodprocessing as emulsifiers, stabilizers and antimicrobial agents (Nitschke and Coast,2007;Muthusamy*et.al*.2008).VanHaesendonck and Vanzeveren,(2004)studied the possibility of using biosurfactantsto improve properties of concentrated cream, hardinity of sweet products, to increase consistency, improving flavor, volume and texture of dough.

The present study focused on the inhibitory effect of rhamnolipids against some microorganisms and probability of applying it in some food products.

MATERIALS AND METHODS

Microorganisms

Pseudomonas aeruginosawas isolated from the contaminated soil ofoil field of NahranUmar in Basra governorate (Swadi,*et al.*,2013).*Klebsiellapneumonia, Salmonellasp., Staphylococcus aureus , Escherichia coli , Micrococcus roseus* were obtained from BasrahHealth Center, *Bacillus subtilis*were obtained from Biotechnology lab./Food Science dept./CollAgriculture .Tow genus of yeast *Candida albicans* and *Rhodotorula* sp. from biotechnology lab./Biologydept./Coll. Science. Rhamnolipids was extracted and purified by AL-Asady, *et al.*,2013in previous study (not published). The bacterial isolates were cultivated in their suitable mediaat 37°C/ 18-24 hr. The yeasts were cultivated in potato dextrose broth at 28°C /24 hr. Determination of inhibitory effect of rhamnolipids and MIC byusing well diffusion method witha concentration 50 mg/ml. The bacteria were spread on Mueller Hinton agar, the incubation was at 37°C/24 hr, whilethe yeasts were spread on PDA,the incubation was at 28°C/48 hr. MIC was determined by using 7.5-15 µg/ml of ramnolipids against *Salmonella sp., Staphylococcus aureus* and *Bacillus subtilis* (Barefoot and Klaenhammer, 1983) .

Detectionof Cytotoxicity of Rhamnolipidson Human Blood

According to the method in(Nair *et.al*.,1989).

Applyingrhamnolipids in food products

- Loaf processing according to (AACC,1976)
- Ice creamaccording to (Nelson and Trout,1964)
- Mayonnaiseaccording to (Marinescu*et.al*.2011)

Statistical Analysis: Using the program (statistical package for social science) (SPSS)17

RESULTS AND DISCUSSIONS

Inhibitory effect and MIC of rhamnolipids: Table (1) revealed the inhibitory effect of rhamnolipids which was

produced by the local isolate *Pseudomonas aeruginosa P.a.28*against some of G+ and G- bacteria and yeasts. The maximum antibacterial activity was against *Bacillus subtilis* with a diameter of clear zone 30 mm and minimum antibacterial activity was against *Micrococcus roseus* with a diameter of clear zone 10 mm, while the inhibitory effect on *Candida albicans* and *Rhodotorula sp.* were with 25 and 17 mm respectively. Figure(1) showed MIC of rhamnolipids was 7.5 µg/ml for *B. subtilis* and 15µg/ml for *Salmonella sp.* and*Staph. aureus* .These results agree with results of Onbasli and Aslim (2009) andGudin*et.al*.,(2010) .

The antimicrobial effects of biosurfactants is due to the structure of biosurfactants which resembled to cell membrane. Biosurfactants are amphipathic molecules with hydrophilic moiety consisting of amino acids or peptides anions or cations; mono-, di-, or polyunsaturated, saturated, or fatty acids. Insertion of fatty acids of biosurfactants into cell membrane caused significant ultra structural changes in the cell such as ability of cell to interiorize plasma membrane (Desai and Banat, 1997; Yalçin and Ergene, 2009).

Table 1: The Inhibitory Effect of Rhamnolipids Produced by the Local Isolate *P.aeruginosa*P.a.28 on Some Pathogenic Microorganisms

Microorganism	Dimeterof Clear Zone(mm)
Bacillus subtilis	30 mm
Salmonella sp	28 mm
Staphylococcus aureus	22 mm
Escherichia coli	20.5 mm
Pseudomonas aeruginosa	18 mm
Klebsiellapneumonia	15 mm
Micrococcus roseus	10 mm
Candida albicans	27 mm
Rhodotorulasp	17 mm

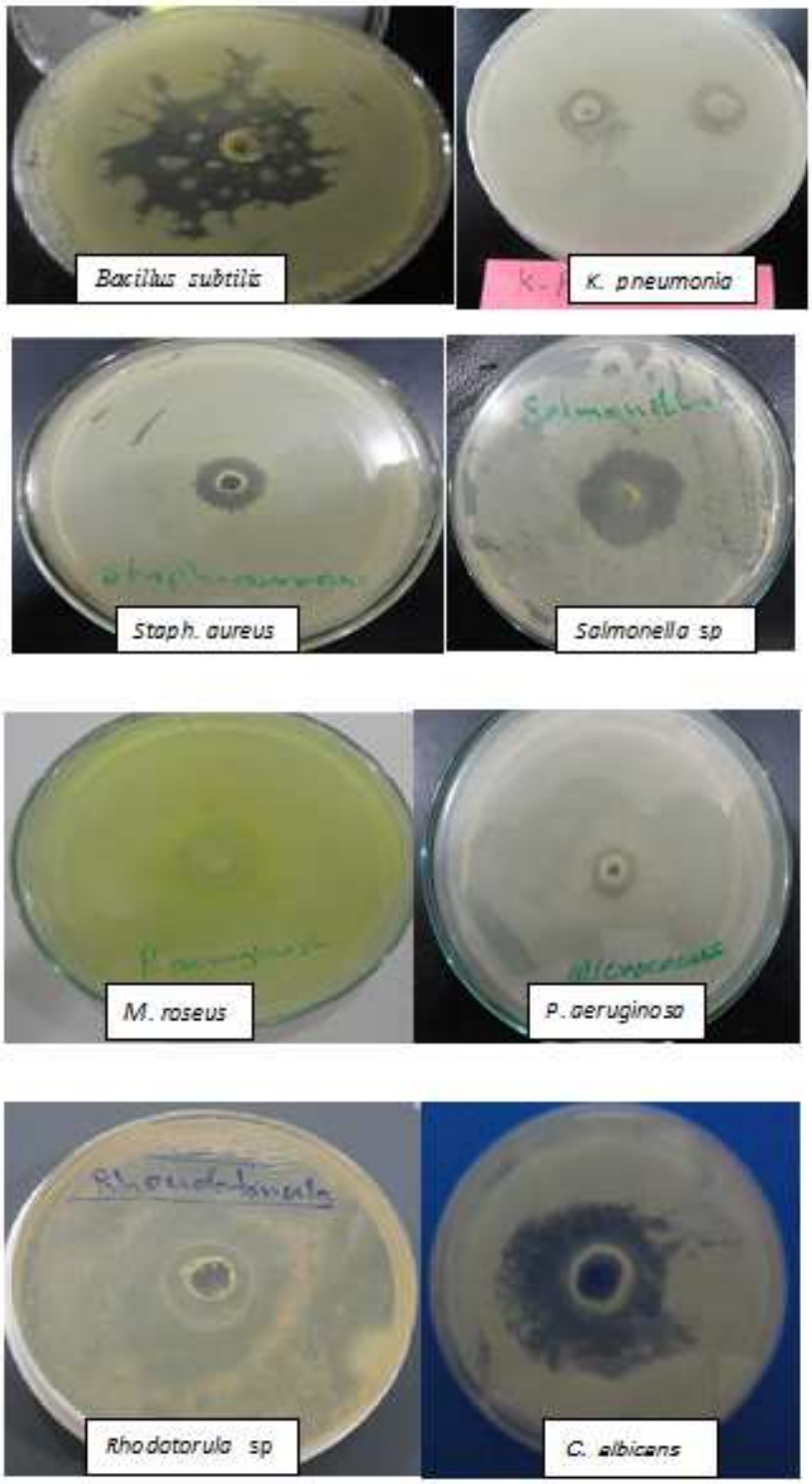

Figure 1: The Inhibitory Effect of Rhamnolipids Which Produced from the Local Isolate*P.aeruginosa*P.a.28 on Some Pathogenic Microorganisms

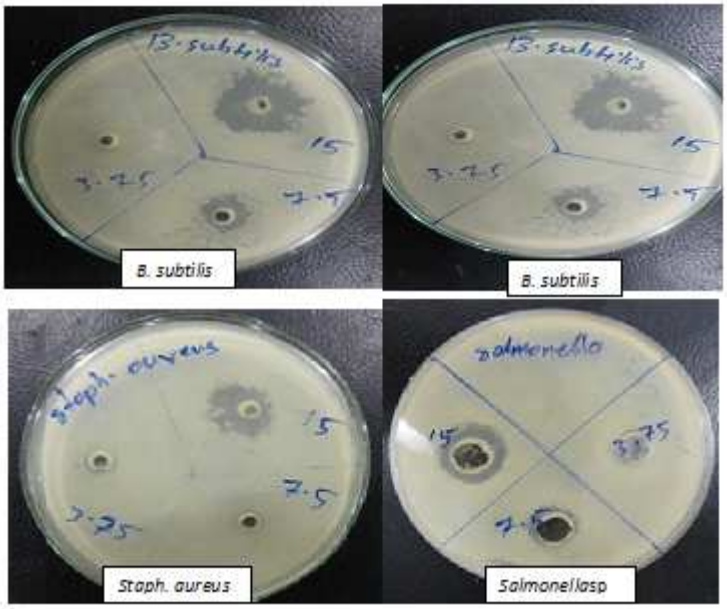

Figure 2: MIC Test of Rhamnolipids WhichProduced by the Local Isolate *P.aeruginosa*P.a.28 on Some Bacteria

Cytotoxicity of Rhamnolipids

Figure(2) revealed that rhamnolipids had no anytoxicity on human blood, The RBC were not precipitated in all concentrations of rhamnolipids and incubationperiods. this means that rhamnolipids have no hemolysis. our results agree with (Nitschke and Coast, 2007;Mazaheri and Tabatabaee,2010)

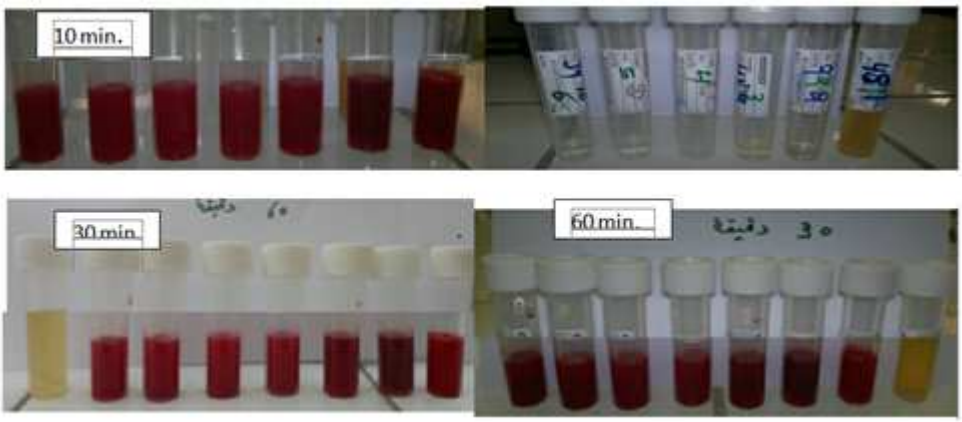

Figure 3: Cytotoxicity Effect Test of Rhamnolipids on Human Blood

APPLICATIONS

- **Loaf Processing**

Table (2) and figure (3) showed the volume of the loaf was improved with 0.7% rhamnolipids because this biosurfactant makes net of gluten more strong and keeping the gas inside the dough. also there are significant differences at level of improbability 0.05 in all properties of the loaf with 0.7 such as the color of surface, smell and taste of pulp and texture. This agree with the results of Van Haesendonck, and Vanzeveren, (2004).

Table 2: The Effect of Applying Rhamnolipids in Loaf Processing

100	15	10	15	10	10	10	3	3	3	3	8	10	Degree of Evolution		Treatment
	Internal Properties						External Properties								
Total	Texture	Munching	Taste of Pulp	Smell of Pulp	Color of Pulp	Graininess	Line of Cut	Similar Body	Baking	Surface	Color of Surface	Score Volume Score	Volume	Weight	
86.9[a]	13.2[a]	8.7[a]	11.5[a]	9.6[a]	9.5[a]	8.6[a]	2.5[ab]	2.8[a]	2.8[a]	7.2[a]	7.7[a]	10[a]	350	145	1
84.9[ab]	13.1[a]	8.3[a]	12.3[a]	7.8[a]	9.4[a]	8.2[a]	2.6[a]	2.5[a]	2.4[ab]	[a]3.2	7.0[ab]	9[b]	310	143	2
79.2[b]	11.8[a]	8.0[a]	11.2[a]	7.3[a]	9.2[a]	8.2[a]	2.0[b]	2.1[b]	2.2[b]	4.2[a]	8.6[ab]	8[c]	300	141	3
69.8[bc]	12.0[a]	7.5[a]	11.6[a]	8.2[a]	9.2[a]	8.7[a]	2.1[ab]	2.5[a]	2.3[cb]	3.2[a]	4.6[a]	7[d]	320	145	4

Treat 1=0.7 rhamnolipids, Treat. 2= 0.5 rhamnolipids, Treat. 3= 0.3 rhamnolipids, Treat. 4= 0.0 rhamnolipids
We used the form in Dalbyand Hill, (1960).

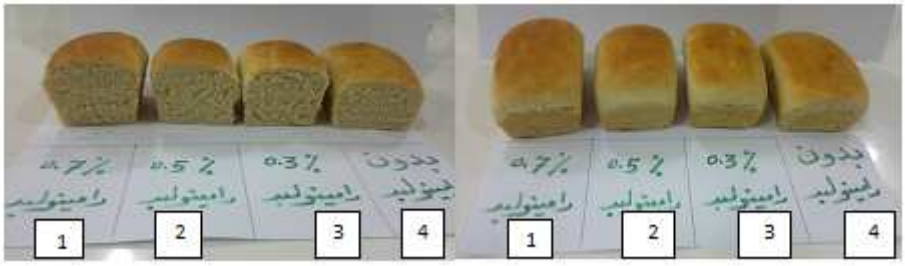

Figure 4: The External Appearance and Profile of Loaf with the Four Treatments of Rhamnolipids

- **Ice Cream**

Table(3) showed the sensory properties of ice cream, there are significant differences at level of improbability 0.05 between treatment 1 with rhamnolipidsas emulsifier and treatment 3 (without rhamnolipids) but no significant differences with treatment 2 (with traditional emulsifier), and the deference's were only in the taste because rhamnolipids improve the taste of food products(Fiechter,1992).

Table3: Effectof Rhamnolipids Applying in Ice Cream

Total100	External Property 10	Color 10	Melting 10	Texture 30	Taste 40	Property Treatment
82.5	8.4[a]	8.6 [a]	8.1 [a]	25.5[a]	31.9[a]	1
80.2	8.3[a]	8.5[a]	8.2[a]	25.2[a]	30[a]	2
72.0	8.2[a]	8.6 [a]	7.9 [a]	25.1[a]	22.2[b]	3

Treat. 1=with rhamnolipids, Treat. 2= control, Treat. 3= withoutrhamnolipids

- **Mayonnaise**

Table(4) and figure (4) showed significant difference sat level of improbability 0.05 between the three treatments, treatment 1 was the best in all properties, it had shiny color and structure with high stability. This is due to the high emulsification activity of rhamnolipids which didn't let oil to separate from mixture, then there was no oily taste in the product(Banat, *et al.*, 2000; Destgheilb, *et al.*, 2008).

Table 4: Effect of Rhamnolipids Applying in Mayonnaise

General Accepting 20	General Appearance 20	Viscosity 10	Texture 30	Taste 10	Color 10	Property / Treatment
16[a]	18.1[a]	9.1[a]	27.1[a]	9.5[a]	9.5[a]	1
14.4[ab]	15.7[b]	7.8[b]	22.7[b]	8.6[b]	8.1[b]	2
12.5[b]	13.2[c]	6.7[c]	19[c]	7[c]	6.9[c]	3

Treat. 1= with rhamnolipids, Treat. 2= with rhamnolipids and egg yolk (1+1), Treat. 3= with egg yolk

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Figure5: Effect of Rhamnolipids on Mayonnaise Properties

CONCLUSIONS

Rhamnolipids which produced by the local isolate *P.aeruginosa* P.a.28 had a wide spectrum for inhibition G^+, G^- bacteria and yeasts, so we can used it in food asa preservative**.** Rhamnolipids had no cytotoxicity**,** and finally this biosurfactant have high emulsification activity which can improve the properties of food products.

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